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JW. MARRIOTT HOTEL
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EXPLORATION OF *ACTINOMYCETES* AS BIOLOGICAL CONTROL OF FUSARIUM WILT DESEASES ON TOMATO AT EAST JAVA

Penta Suryaminarsih and Tri Mujoko

ABSTRACT

This study aimed Actinomycetes as the biological control of Fusarium wilt deseases on Tomato. The research was conducted at plant Protection Laboratory and Glasshouse, Faculty of Agriculture, Pembangunan Nasional "Veteran" University started from April trought November 2008.

In the Laboratory the antagonism test was applied to the result of actynomycetes isolated on site using the Complete Randomized Design with three replication. A total of 8 actinomycetes isolated on site were tested in terms oftheir inhibition potential toward Fusarium. Antibiotic testing was applied to the potentialisolates using disc paper agar and water agar. The parameters observation were the inhibition zone, colony size of Fusarium.

The glasshouse research used the Complete Randomized design with 4 replications. There were the actinomycetes isolates, namely the pinus, corn, hot chili and tomato on pare isolates. The parameters were plant infection percentage, plant height and actinomycetes at the end of the experiment.

On site isolated yielded 8 actinomycetes isolates : 5 from Malang(one from pine, one from corn, one from hot chili and two from tomato), 3 from tomato Pare. For isolatres had the antagonist potential and inhibited the Fusarium growth. The pine, corn, hot chili isolate from Malang were antibiosiswhereas the pare isolates was competitive. The identification of 5potential isolates include the streptomyces genus.

At the screenhouse research not shown wilt infection until third weeks. Despitefully isolate from pine, corn, and hot chili indicate as PGPR.

Key words : *Fusarium oxysporum*, Tomato, Biology control, *Actinomycetes*

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Mulyorejo, Surabaya, East Java, IndonesiaPhone and Fax 06231 5992445

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I. Introduction

To fulfill the needs of horticultural product particularly in the country and export, it is needed an effort of development horticultural commodity that refer to increase of trade efficiency, the productivity through mastery of IPTEK and the others. The increase of tomato often get there are many obstacles, one of them is fusarium disease. In East Java the average of fusarium attack is 10,12%, nevertheless four of five planting areas that observed found the fusarium. The observers noted that in Wajak (Malang) this pathogen attack could reach for about 30%.

One of alternative way to overcome the fusarium and to keep balancing land microorganism is usefulness of natural enemy by the right way in order to save for the environment. The use of actinomycetes directly as life agents could control the fusarium of Tomato farm glasshouse scale (Mujoko, 2005), nevertheless for the application in field is rare to be observed. Therefore it is needed to conduct a research about the potency (ability) kinds of isolate from the kinds of plants in East Java and the way of actinomycetes application to control fusarium of tomato in farm. Hopefully the result of potential isolate could overcome the obstacles of fusarium on tomato and other area.

II. Research Method

This research applies some experiments as follows.

Fusarium isolate. The method used for fusarium isolate is use spore stimulation from fresh material (Sastrahidayat, 1994). Those pathogens are isolated from tomato from field (Wajak-Malang) that attacked by fusarium. The part which shows the symptom disklorasi is cut flimsy aseptically. Those cut is noculated on selective medium for fusarium.

Actinomycetes isolate. Actinomycetes is got in tomato farm which not get symptom of fusarium attack pine forest, the sample took from pine root with

deepness 5-15 cm in Batu, Karangploso, Wajak, and from Pare and Wates Kediri. Actinomycetes isolate is used by medium Glucose Nitrate Agar (GNA), where the mushroom and bacterium can not live in this medium (Sastrahidayat, 1994 b) by the dilution plate method.

The examination of antagonism in Laboratories

Inokulum actinomycetes or Fusarium, are took from breeding in the form of cutting inokulum by using drill cork diameter 0,5 cm. those both microorganism are placed in a solder cup which filled with PDA. After the incubacy for 2-3 days in chamber temperature, the radius growth of fusarium colony is measured everyday (the observation stopped after pathogen touch the solder cup). The resistance of growth colony expressed in percentage which counted with formula (Van den Heuvel, 1970):

$$Mi = \frac{Mc - Mt}{Mc} \times 100$$

Mi = the percentage of resistance
Mc = growth in control
Mt = pursued growth

The examination of antibiotic

This examination is conducted by two ways, they are:

a. Diagram of paper disk. The examination use filter paper disk 0,5 cm which have been soaked into conderasation antibiotic which got by: ampoule bottle 20 ml filled with liquid media AFM as much 10 ml. after that the bottle is noculated with actinomycetes isolate as much 1 piece of isolate diameter 0.5 mm, after that it is incubated. Kultur which is growth for a few days (for about 14 days) in the ampoule bottle filled with liquid media AFM then sipped the air by using spite 10 ml. the condensation which contain of antibiotic is saved in refrigerator (temperature 4° C). And then the effectiveness of this antibiotic is examined by filter paper disk then dried. The disk which contain of antibiotic is noculated soon on medium PDA in a solder

cup which have been noculated suspense spore test fusarium before (figure 1). The zone resistance which caused by filter paper disk and fusarium colony represent the indicator of resistance and the diameter is measured as mentioned above.

b. Cellophane. Ground of medium of soil chitin (250 g) placed in plastic bag heat resistant (pp), is sterilized in autoclave. Aseptically the ground is noculated with actinomycetes isolate diameter 0.5 cm as much 5 cuttings and is incubated for 21 days. After that one-third part ofthe ground poured in a sterile solder cup. After compacted, the medium water agar (45° C) poured on top of the ground slowly in order to flatten. After becoming soild, the top of the ground is covered by coat of cellophane, and on the top of this coat right in the middle of cup noculated inokulum pathogen test by using cutter biscuit diameter 0,5 cm. the observation is conducted on wide of colony fusarium.

The identification conducted by using standard from Bergey's Manual of Determinative Bacteriology (Hoelt et all., 1994). For identification, the isolate is breaded on agar slide, in order to easy to recognize the form of colony morphology and the conidium's. Gram coloration is conducted to differ with other microorganism.

III. Findings and Discussions

From the result of observation explorasion and antagonism test in laboratories found four main candidates (figure 3) which could pursuing mushroom fusarium oxysporum cause wilt disease on tomato farm table 1.

Table 1 Result of Antagonism examination some candidates of Actinomycetes from tomato, chili, pine and corn field in Pare and Malang

No.	Nature of Candidate	Colony Morphology	Resistivity	Boldness
1	Chili field, Pare	Bright red, solid and thick	1,3 cm	Strong
2	Corn field, Malang	Yellow grey thick less solid	1,2 cm	Strong
3	Tomato field, Pare (TP3)	Dark grey, less solid, thick	Only static-function, 0,6 cm	Medium
4	Pine field, Malang	White chocolate, solid and thick	1,3 cm	Strong

Species difference is anticipated as the cause of diameter of resistivity zone differences so that the antibiotic resulted is also different. According to Kim, Moon and Hwang (1999) tell that antibiotic potency influenced by strain, condition of medium breeding and concentration. Also condition of medium breeding with full nutrition will cause the high production of antibiotic than medium of poor nutrition (Betina, 1983). Among actinomycetes, Genus Streptomyces will produce the high antibiotic at medium with rich nutrition as the source of carbon and high nitrogen besides mineral (Stanbury and Whitaker, 1984). Besides the explanation above, the breeding age and pH according to Chang et al (1981) also influence production of antibiotic.

Table 2. Resistivity Percentage of Any Kinds Isolate Actinomycetes toward Mushroom F. oxysporum

No.	Isolate		Resistivity (%)
1	(Red) chili	Tm	**51,5**
2	Corn (yellow grey)	Tm	**37,5**
3	*Pine (red fanta)*	Tm	**51,7**
4	Pare Tomato (grey)	1	5
		2	10
		3	**21,7**
5	Malang Tomato	1	5
		2	10

Isolate with strong energy, more than 1 cm and or mechanism of competition antagonism used to later test. From table 1 and 2 there are three isolates from chili, corn and pine field which has strong resistivity more than 1 cm from chili, corn and pine field later called as chili isolate, corn isolate and pine isolate. For the isolatee of Pare tomato field, although the resistivity is low but since it causes the growth of thin miselium Fusarium and mechanism of competition antagonism is also used as later test.

2. Antibiosis Test Paper Disk Media

The result of antibiosis test paper disk media shows that Actinomycetes from chili is stronger than other isolate. The immersion of bloter paper in antibiotic fluid will cause the antibiotic absorb into the paper. Thus, if the paper is put down on the media consist of fusarium, the free zone will appear around the paper. The wide of

the zone shows antibiotic strength rate of actinomycetes. For the antibiosis test paper disk, it is need to be incubated for 7 days.

Table 3. Average Diameter of Resistivity Zone Actinomycetes toward F. oxysporum on the paper disk media

Treatment	Diameter (cm)	Category
F x AT	0.5	Medium
F x AC	1.2	Strong
F x AP	0.4	Medium
F x AJ	0.2	Weak

3. Antibiosis Test Semi Natural in Vitro Media

The ability of resistivity and assumption about antibiotic are also can be detected through semi natural breeding that is through breeding at water jelly. The observation toward Fusarium diameter shows that the test isolates are able to press the growth of Fusarium. The result on this observation shows antibiosis test on the water jelly media.

Table 4. Average Diameter F. oxysporum on water jelly media which consist of Isolate Actinomycetes

Treatment	Colony Diameter (cm)
F x AT	0.73 b
F x AC	0.6 ab
F x AP	0.5 a
F x AJ	0.6 ab
Control (F)	3 c

Actinomycetes having antagonist character toward Fusarium is caused by actinomycetes has ability to result secondary metabolit with antibiotic character. The same condition reported by Gottlied (1973 in Sykes and Skinner, 1973) that Actinomycetes is the greatest antibiotic producer that is 85%, followed by mushroom 11% and bacteria 4-5 %. Not all the actinomycetes can produce antibiotic, among the actinomycetes genus Strepomycetes is the greatest antibiotic contributor. There is active Antibiotic, produced by Actinomycetes, which fight the bacteria and mushroom (Nolan and Cross, 1988).

IV. CONCLUSION

There are four (4) isolates pursuing the growth of Fusarium oxysporum in vitro. Chili isolate, corn isolate and pine isolate are antibiosis, 1 tomato isolate from Pare is static-function.